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"HETEROTYPICAL" MITOSIS IN NEREIS LIMBATA (EHLERS).

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As the result of investigations on the chromosomes in *Enteroxenos östergreni* I published some years ago (Bònnevie, 1905, 1906) the following conclusions (p. 384, 1906):

"Die Zahlenreduktion der Chromosomen geschieht bei Enteroxenos durch ihre parallele Konjugation in Synapsis. Die dadurch entstandene Doppelheit der Chromosomen geht weder in der ersten noch in der zweiten Reifungsteilung wieder verloren, sondern tritt noch in den Vorkernen deutlich hervor und verschwindet erst im Laufe der folgenden Zellgenerationen mit der völligen Verschmelzung der konjugierten Chromosomen.

"Die konjugierten Chromosomen haben ihre Teilungsfähigkeit behalten; beide Reifungsteilungen sind somit als Aequationsteilungen zu betrachten, deren Bild jedoch durch die Doppelheit und die Grösze der Chromosomen kompliziert wird. Das rasche Aufeinanderfolgen beider Teilungen trägt zu einer Grószenreduktion der Doppelchromosomen bei; sie werden jedoch erst im Laufe vieler Zellgenerationen auf ihre ursprüngliche Grösze reduziert."

At the time when I first formed this opinion, no clear evidence existed of the corresponding process in other organisms sufficient to make my conclusions seem improbable. But during the time which passed before the publication of my final work a series of papers (Schreiner, 1904, '05; Grégoire, 1904, '05; Montgomery, 1905) appeared, which — though from different points of view — all agreed in claiming for the first maturation mitosis the general significance of a reductional division, separating the chromosomes, which had conjugated at an earlier period.

Opposed to the apparently strong evidence in favor of a reduction division brought together in these papers, as well as that from the valuable investigations of Schreiner (1906*a*) on the maturation process in *Tomopteris* — my results on *Enteroxenos* stood quite isolated. And the reasons which at an earlier period had seemed strong enough to support my view, might now seem inadequate.[1] Even before the appearance of my final paper (1906) I therefore felt the necessity first of reinvestigating the maturation process in *Enteroxenos* and second, in case of the confirmation of my earlier results of finding another object, in which the behavior of the chromosomes might be more easily followed than in this species.

On reinvestigating my old *Enteroxenos*-preparations as well as new material of the same species, I found, that although it might well be possible, even in this species, to select a series of maturation stages showing the "*Tomopteris*-type" (Schreiner, 1906*a*), yet other structures are present in the chromosomes which do not support the assumption of a reduction division.

Besides the great similarity in the general appearance of the two maturation divisions there is a longitudinal split in the chromosomes at the end of this period — a structure which suggests an interrogation as to the assumption of a reduction division, until the existence of this mode of mitosis has been proved for this very species.

On the other hand, however, I willingly admit that *Enteroxenos* is not a favorable object for a decision of these difficult questions — the chromosomes being very much contracted during the metaphase and so small, that the structures in question are often beyond the limit of an objective demonstration. It therefore seemed desirable to extend my investigations to other species with more favorable chromosome relations.

[1] (Added on the proof-sheet, June, 1907.) The truth of this sentence was clearly proved through the appearance of the latest paper by A. and K. E. Schreiner (1907) some weeks ago. Their results seem to prove that "the new observations in my paper were not good, while the good ones (if present) were not new." I hope however, through this and my following publications to show that my main results on the maturation divisions in *Enteroxenos* were correct, and that the doubt which they made me feel with regard to the existence of a reduction division in this species was well founded, even if future observations should show that my interpretation of the new facts would have to be modified.

At Columbia University, New York, where I have spent last winter, I have had the best opportunity of doing this; and I want here to express my most sincere thanks to Professor E. B. Wilson for offering me a table in his laboratory, for his generous liberality in giving me free use of his valuable material, and for the lively interest with which he has followed my work.

In this paper I wish to give a preliminary account of my results on *Nereis limbata* Ehlers, a species in which the chromosomes are especially favorable for an investigation of the mitotic process — results which have obliged me to maintain a position different from that represented in the papers of Grégoire and Schreiner.

The most important of these papers, it seems to me, is that of A. and K. E. Schreiner on *Tomopteris* (1906*a*). In this species they have found an object, in which the whole maturation process of the chromosomes can be followed and demonstrated with an apparently indisputable clearness. After a comparison of their results on *Tomopteris* with the maturation process in other animals and plants (1906*a* and *b*), they find it very probable that (p. 474, 1906*b*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird, das ihm unter ähnlichen Verhältniszen ein ähnliches Gepräge aufdrückt, und zwar das Gepräge des '*Tomopteris*-Typus'"; and also that (p. 475) "die Zeit nicht fern ist, wo das 'Reduktionsproblem' von morphologischem Gesichtspunkte aus als gelöst angesehen werden darf."

I fully agree with A. and K. E. Schreiner, that the knowledge of the maturation process in *Tomopteris* is of great importance for our understanding of the same period in other organisms. Especially valuable seems to me their convincing demonstration of a parallel conjugation of the chromosomes in this species and their identification of the same process in so many other groups.[1] Of great value also are the demonstrations of Grégoire

[1] I have reason to believe that the conjugation process in *Enteroxenos* follows the type of *Tomopteris* more closely, than is shown in my figures (Bonnevie, 1906, fig. 33–42 and 159–162). During my first investigation of the spermatocytes of this species I both observed and figured stages, in which there was a parallel arrangement of thin chromatic threads, the ends of which were directed towards one pole of the nucleus; and it was, in fact, these pictures which made me join von Winiwarter

(1905) and of Schreiner (1906*a* and *b*) of the close resemblance between the chromosomes of very different species during the first maturation mitosis; and certainly, the presence of this same type throughout the whole animal (and plant) kingdoms cannot but give one the impression that (Schreiner, *loc. cit.*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird."

But what is the law that determines the behavior of the chromosomes during the maturation period?

In my opinion the answer to this question is not yet given, in spite of the apparently overwhelming evidence brought together in the works of Grégoire and Schreiner to demonstrate that the first maturation division is always a heterotypical one, and that this heterotypical character finds its explanation in the fact, that (Schreiner, 1906*a*, p. 44) "hier Ganzchromosomen, die nie mit einander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

My results in *Nèreis* will, however, clearly demonstrate that a "heterotypical" mitosis cannot be considered as identical with a reductional one; and I hope to show in the following pages that the problem of the reduction of the chromosomes is still entirely open to discussion.

My material consisted of a most valuable series of maturation and segmentation stages of *Nereis limbata*, collected by Professor E. B. Wilson at Woods Hole, in 1896–97. The sections as well as the uncut material, fixed partly in picro-acetic acid, partly in Flemming's fluid, proved to be still in perfectly good condition; and the material contains an uninterrupted series of stages, from the moment of fertilization to the four-cell stage, and also the later segmentation stages, up to fifteen and one half hours after fertilization, these, however, with intervals in their development of two to five hours.

(1901) in his hypothesis of a parallel conjugation. As I, however, found very few cells of the same appearance among the young oöcytes, I did not believe this to be the typical arrangement of the chromatin; and I therefore tried to find other stages showing the first traces of a parallelism between the thin threads. In the light of the chromosome relations in *Tomopheris* I now think it probable, that a reinvestigation of new material of *Enteroxenos* will give results somewhat different from those shown in my paper. (Added June, 1907.) This supposition is confirmed by Schreiner, 1907.

The maturation process in the egg of *Nereis* does not begin until fertilization has taken place, and the earliest stages contained in my material show the nuclear membrane still unbroken, while outside of it two small asters have made their first appearance. Within the large nucleus fourteen chromosomes are found scattered around, most of them, however, lying relatively near to the nuclear membrane.

The chromosomes appear in shapes, well known from other worms — *Allolobophora* (Foot and Strobell, 1905), *Tomopteris* (Schreiner, 1906*a*) and others — forming rings and crosses of different kinds; but they also very often appear in a more irregular shape. (See earl. proph. of 1st mat. div.; p. 62.)

A comparison of these different chromosome forms shows

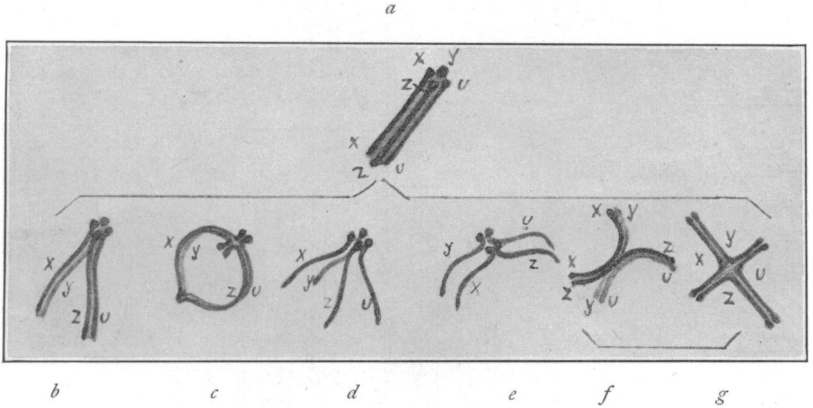
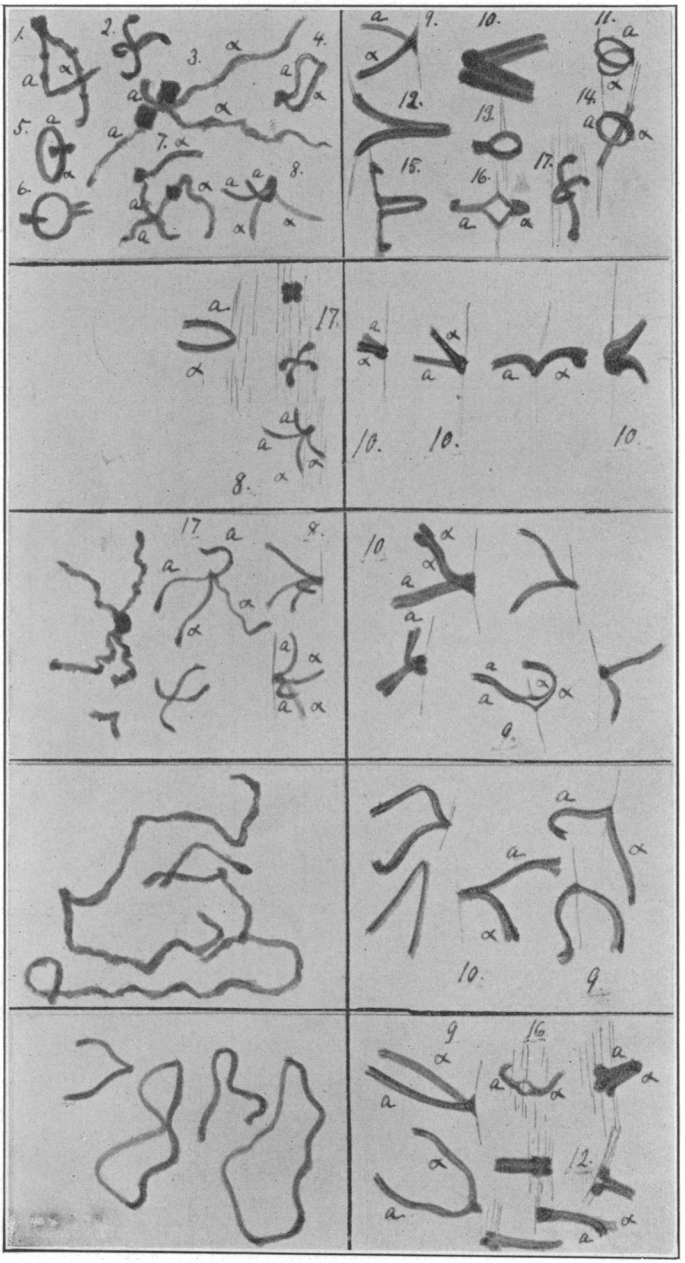


FIG. 1, *a–g*. Schematic illustration of the development of chromosomes from the original tetrad. Explanation in the text.

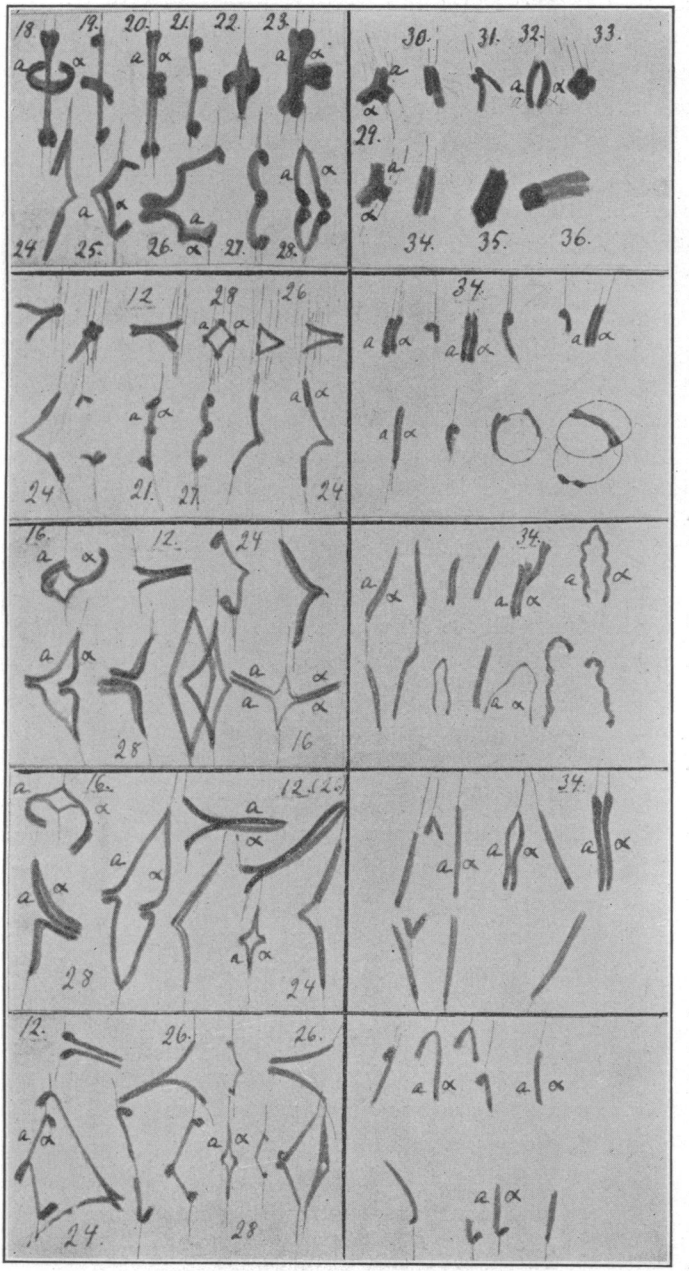
that they all are reducible to one and the same type — to a more or less elongated tetrad (Fig. 1, *a*) in which the four originally parallel elements may be arranged in different ways (Fig. 1, *b–g*).

In most cases the four elements are combined in pairs, so as to give the appearance of two longitudinally split ribbons, connected at one (Fig. 1, *b*; chrom. 1, p. 62), or at both ends; in the latter case the chromosomes form more or less typical rings (Fig. 1, *c*; chrom. 4–6, p. 62).

In other tetrads we find the four elements connected at one end, but diverging from this point in different directions (Fig. 1, *d*). Such an arrangement gives rise to cross-shaped chromo-


Early Prophase
Later Prophase
1st Maturation Division
2nd Maturation Division
Early Cleavage Divisions (1st–3rd)
Cleavage Divisions 7½–11 h. after Fertilization.
Cleavage Divisions 11–15½ h. after Fertilization.

Early Anaphase | Later Anaphase



1st Maturation Division

2nd Maturation Division

Early Cleavage Divisions (*1st–3rd*)

Cleavage Divisions 7½–11*h.* after Fertilization.

Cleavage Divisions 11–15½ *h.* after Fertilization.

somes with four single arms, all of the same length and usually extending to one and the same side of their connecting point (chrom. 3, 8, p. 62).

Another kind of cross — double-armed ones — may also be derived from the original tetrad, its four elements being arranged in pairs, but combined in a different way at each end of the tetrad (Fig. 1, *f*. At the upper end the elements $x + y$ are diverging from $z + u$, at the other end $x + z$ remain parallel, diverging from $y + u$.) Through a flattening down of a figure, formed in this way we get a cross-shaped chromosome (Fig. 1, *g*), whose arms appear longitudinally split, and in which always two arms, lying opposite to each other, are of the same length. Sometimes this may be the case with all four arms, but more often we find a considerable difference between the two pairs. In many chromosomes this difference is so great that the short arms of the cross appear only like a pair of lateral projections on the middle of a longitudinally split ribbon; and from those forms there is a very short step to the chromosomes represented in Fig. 1, *b* and *c*.

Finally we may often find chromosomes consisting of two apparently separate halves (Fig. 1, *e*; chrom. 7, p. 62). Here also the origin of the chromosome may be traced back to a tetrad, and most easily through a transition form like that in Fig. 1, *b*, the arms of such a chromosome being divided along their longitudinal split.

A comparison of the whole chromosome group in a number of nuclei shows that these different forms are not characteristic of special chromosomes. I have found nuclei, in which all the chromosomes were cross-shaped, others in which two or three rings were present among the crosses, and again others, in which one or both of these forms were mingled with the more irregularly formed chromosomes.

Nor did I find any evidence in favor of the view, that the different forms of the chromosomes should represent different stages in their development. It seems more probable that the rings, the two kinds of crosses, the rodlike chromosomes, etc., arise simultaneously from the original tetrads and that their special shape is more a result of chance — possibly of their conditions within the nucleus — than of any individual character of the

chromosome. The formation of rings seems, however, to be limited to chromosomes of a certain size.

At the time of the dissolution of the nuclear membrane the chromosomes are found to be slightly contracted, and while some of them regain their original tetrad-form, others remain like V- or horseshoe-shaped rods, longitudinally split and with a thickening at their middle point. Also the ring-shaped chromosomes usually retain their form, while the numerous crosses, found within the nucleus, are transformed into tetrads or V-shaped chromosomes. In only one case were two cross-shaped chromosomes found attached to an early spindle.

Considering the chromosomes of the prophase as different modifications of the tetrad, we will find that their attachment to the spindle-fibers is in all cases a terminal or a slightly subterminal one. (See lat. proph. of 1st mat. div., p. 62).

The unmodified tetrads are attached at one end, their longitudinally split halves being separated from each other (chrom. 12, 15).

The V- and horseshoe-shaped chromosomes are attached at their middle point, this representing one end of the original tetrad (chrom. 9, 10, 16).

The rings are placed horizontally [1] on the spindle and the fibers attached either at their transverse projections (chrom. 14) or at the point opposite to these (chrom. 11).

In each case the attachment is a terminal one, and the rings, as also the V-shaped chromosomes, are divided along a plane represented by their longitudinal split. In the above mentioned case in which I have seen cross-shaped chromosomes attached to the early spindle, the point of attachment seemed to be at their center, all four arms being bent in a direction away from the axis of the spindle (chrom. 17, p. 62). Considering the crosses as tetrads with four diverging elements, we find here also a terminal attachment of the fibers.

Besides the forms already mentioned I also found, in two or three cases, ring-shaped chromosomes placed in such a way on the spindle that they must be divided into two half-rings (chrom. 13, p. 62). In all of these cases, however, the rings were smaller

[1] In the following description the axis of the spindle is always supposed to have a vertical position.

than those found within the nucleus, and which we have seen placed horizontally on the spindle. I consider, therefore, that this is undoubtedly a secondary ring formation, caused by a subterminal attachment of the fibers to a tetrad-shaped chromosome.[1]

The early prophase, in which the chromosomes are yet quite irregularly scattered on the surface of the spindle, is, according to the above stated facts, characterized by an arrangement of the chromosomes at right angles to the axis of the spindle.

This stage is followed by another, in which this horizontal position of the chromosomes is gradually changed into a vertical one, the daughter chromosomes being pulled towards each pole of the spindle (see earl. anaph. of 1st mat. div., p. 63).

During the time in which this separation takes place the chromosomes very often pass through a second cross-like stage (chrom. 18, 19, 22), the fibers being attached at or near the ends of the vertical arms of the cross. At first the horizontal arms are relatively long; a longitudinal split may be clearly visible in them, and they are often bent (in a direction away from

[1] The behavior of the ring-shaped chromosomes in *Nereis* confirms my conclusion from *Enteroxenos* (Bonnevie, 1905, 1906) that the rings of the prophase cannot always be considered as identical with those of the metaphase. The prophase rings are in *Nereis* divided in their own plane, and during the separation of the daughter-chromosomes other rings are transiently formed from tetrads and V-shaped chromosomes; these metaphase rings are then sooner or later divided into two half rings.

According to a note in their latest work A. and K. E. Schreiner (1906*b*, p. 442) seem to have observed metaphase-rings in the first maturation division of *Enteroxenos*. This fact does, however, neither change nor contradict my results on the same species, that other rings are divided in their own plane and thus still appear as rings in the telophase.

In the same paper (Schreiner, 1906*b*, p. 444), is found the following phrase:

"Die Verfasser (Farmer u. Moore) meinen jetzt, wie Montgomery und Bonnevie, dass die bivalenten Chromosomen nicht durch Spaltung der in reduzierter Zahl vorhandenen Schlingen, sondern durch Zussammenbiegung derselben gebildet werden."

Because of this misleading account I want here once more to state my exact position with regard to these questions. I have described the bivalent chromosomes arising through a parallel conjugation of two homologous univalent ones — a view which is very different from that held by Montgomery and by Farmer and Moore. And with regard to ring-shaped chromosomes, I have shown that they may be formed in different ways, through an approach of the free ends of a chromosome (postsynapsis of *Enteroxenos*), or through a widening of a longitudinal split, while the two halves of the chromosome are still connected at their ends (cleavage division of *Enteroxenos* and many cases described in the literature). And further, that from the presence of ring-shaped chromosomes no conclusions can be drawn with regard to the nature of the mitosis, as it has been shown that rings may divide in two different planes.

the axis of the spindle) so as to form a more or less nearly closed ring (chrom. 18, 22).

The vertical arms of the cross on the other hand, are at first short, and their elongation evidently takes place at the cost of the horizontal arms. They are in most cases thinner than the horizontal arms (except their endpiece, if this is lying beyond the point of attachment of the fibers), and very often no longitudinal split can be seen in this part of the chromosomes (chrom. 19, 21, 24, 27). It is clear, however, from their earlier as well as from their later stages, that a doubleness is present even here; and when it is not visible, it may be due to the stretching of the vertical arms of the chromosomes.

Metaphase rings seem to be formed mostly from horseshoe-shaped chromosomes (chrom. 9, 16, 28). They are divided into two half rings. I have never observed a longitudinal split in these half rings; and an examination of a great number of chromosomes shows that the metaphase-rings of *Nereis* are to be directly compared with the cross-shaped chromosomes of the same stage, the space between the two branches of each half ring being identical with the longitudinal split in the vertical arms of the cross. Very often we find this space in the rings filled by an achromatic substance (like the "Zwischensubstanz" of the chromosomes of *Enteroxenos*);[1] and we find, indeed, all transition stages between the open rings and the thin vertically stretched arms of the crosses.

[1] (Added on the proof-sheet, June, 1907.) The existence of such a substance in the chromosomes of *Enteroxenos* is absolutely denied by A. and K. E. Schreiner (1907). They say (p. 12):

"Wir haben an unseren Präparaten vergebens nach dieser sehr eigenthümlichen Substanz gesucht, und es scheint uns unzweifelhaft, dass sich Bonnevie in diesem Punkt vollkommen getäuscht hat, indem sie bald die Spalte zwischen den Komponenten eines Doppelchromosoms, bald die Längslichtung in den Komponenten selbst, bald aber auch achromatische Verbindungen zwischen zwei oder mehreren Chromosomen als eine Kittmasse gedeutet hat."

In the face of this sweeping criticism I can only repeat that in *Enteroxenos*, as well as in *Nereis*, *Thalassema* and *Doris*, I have found the chromosomes of the maturation divisions, and especially those of the first, containing an achromatic substance which can be stretched out to a considerable width between the branches of the chromosomes.

In this respect the chromosomes of the maturation differ from those of other divisions in the same material.

If this substance has not been visible in Schreiner's preparations, it must depend upon its being dissolved or contracted.

After their separation the daughter chromosomes contract to relatively short and thick rods, in which the longitudinal split is in most cases clearly visible (see p. 63, lat. anaph. of 1st mat. div.). Sometimes the two halves of these chromosomes show a tendency to diverge from each other at their free end (chrom. 31); and once I have found a pair of daughter chromosomes (chrom. 29) in which a double longitudinal split seemed present — the one which is usually visible (separating a from a) and on the inside of the diverging halves of the chromosomes another split at right angles to the first one.

Such a tetrad-like appearance of the daughter chromosomes is found more often in the telophase (chrom. 35, 36). But I have not been able to decide with certainty, whether or not this appearance is due to a mere surface structure. An examination of the following stages, however, makes it very probable that these chromosomes ought to be considered as real tetrads.

Reviewing the different stages of the first maturation division, we find:

That the point of attachment of the daughter chromosomes corresponds to a point at (or near) the end of the original tetrad.

That the plane of division was represented by the longitudinal split of the rings and the V-shaped chromosomes of the early prophase — and

That the longitudinal split of the daughter chromosomes is identical with the space between the two arms of the V-shaped chromosomes.

What has been said about the chromosomes of the first maturation division may with some modifications be applied also to any of the following divisions up to fifteen and one half hours after fertilization.[1]

The elongated chromosomes are before each division placed horizontally on the (vertical) spindle, and — transiently appearing like rings, crosses, E-shaped chromosomes, etc., — they are before the separation of the daughter chromosomes carried into a position parallel to the spindle fibers.

[1] Whether the same type of mitosis may be found throughout the whole life of the animal, I am not yet able to say.

Up to eleven hours after the fertilization a longitudinal split may be clearly visible in the terminally (or subterminally) attached daughter chromosomes, this split being as in the first maturation division identical with the space between the two branches of a metaphase-halfring or, which is the same, between the two diverging arms of the horseshoe-shaped chromosomes of the prophase.

With regard to this period, therefore, it will be enough to mention the characteristics through which each division, or group of divisions, is distinguished from the other ones (comp. the chrom. of the different divisions on pp. 62–63).

Second Maturation Division.

In the eggs of *Nereis* there is no resting stage between the two maturation divisions, the formation of a new spindle taking place even before the first polocyte is fully separated from the egg-cell. The chromosomes, therefore, pass directly from the telophase of the first division into the prophase of the second, and no great changes are seen to take place in their structure (see p. 62, 2nd mat. div.).

The chromosomes of the prophase are mostly V- or horseshoe-shaped with a longitudinal split and attached by their middle point. There are however also found cross-shaped ones with four equally long arms being attached by their center on the surface of the spindle. In the metaphase we find the chromosomes arranged in a circle round the equator of the spindle, their form being through an approach of the diverging arms, transformed into a rodlike one. In a few cases I have seen a tetrad-like structure of these chromosomes (chrom. 12, p. 63) a fact which is in complete harmony with their genesis and with the appearance of a longitudinal split in the daughter chromosomes.[1]

[1] (Added on the proof-sheet, June, 1907.) With regard to a similar doubleness of the chromosomes shown by me (1905, '06) in *Enteroxenos*, A. and K. E. Schreiner express themselves as follows (1907, p. 18):

"Weder die Beobachtungen Bonnevie's von dem Vorhandensein einer solchen Doppelheit der Chromosomen, noch ihr Versuch, dieselbe mit der in der I. Reifungsteilung sichtbaren zu vergleichen, sind neu; vielmehr gibt es schon über diese Frage eine ganze kleine Literatur, die aber von Bonnevie nur geringe Beobachtungen gefunden hat."

They then mention the observations of Ed. van Beneden (1883) and Flemming

In the telophase the chromosomes grow longer and thinner, their longitudinal split often disappearing; and small drops of hyaloplasm are seen accumulating at the side of each of them, or between two neighboring chromosomes (lat. anaph. of 2d mat. div.). Through the growth and fusion of these vacuoles the female pronucleus[1] is formed; and the chromosomes, invariably adhering to the surface of the vacuoles, soon lose their staining power so that they cannot be distinguished within the resting pronucleus.

Early Cleavage Divisions.

In the early prophase of these divisions the chromatic substance of the nucleus appears in form of an irregular network, which, however, soon proves to consist of a number (28) of cross-shaped chromosomes, each with four equally long arms without any longitudinal split, and many of them with a conspicuous thickening at the center (earl. proph. of earl. cleav. div., p. 62).

These crosses, attached on the young spindle by their middle-point, are in later stages transformed into V-shaped, longitudinally split chromosomes — a transformation, which can only be due to an approach of two arms of the primary crosses on each side of their point of attachment.

The metaphase of these divisions differ from the maturation di-

(1887), both referred to in my final paper (1906, p. 390),—and besides these also those of Hof (1898) and Merriman (1904) on vegetative cell-divisions in plants.

According to observations, to be published in a following paper, I can now say, from my own experience, that this doubleness, occasionally mentioned as occurring outside of the maturation period is (with exception perhaps of Van Beneden's observations in the segmentation divisions of *Ascaris*), something quite different from the doubleness of the chromosomes at the end of the maturation period first found by me in *Enteroxenos* — and now also in *Nereis*, *Thalassema* and *Doris*.

What Hof (1898) has seen in "den eben fertig gebildeten Tochterkernen," is the same structure, which is later described by Merriman (1904). She, however, neither has, nor pretends to have, seen a real doubleness of the daughter chromosomes; and her comparison with the maturation phenomena consists in suggesting that also the doubleness so often described at this stage should be (p. 202) "due to the changing of the daughter-chromosomes from tubular structures into the quadripartite threads."

I must, therefore, insist upon the priority of having shown a doubleness of the chromosomes at the end of the maturation divisions. After the appearance of my preliminary account (1905), however, similar structures were shown to exist in *Myxine* (Schreiner, 1905), in *Ascaris mystax* (Marcus, 1905) and in *Dytiscus* (Schäfer, 1907).

[1] The male pronucleus also develops at the same time and in a similar way.

visions, as well as from the later cleavage divisions, practically all the chromosomes retaining their V- or horseshoe-shape and accordingly also their median point of attachment on the spindle (lat. proph. of earl. cleav. div., p. 62). In the early anaphase we therefore here find a greater number of ring-shaped chromosomes than in any of the other divisions.

The aspect of the later anaphase seems at first to form a striking contrast to that of earlier stages, the daughter chromosomes now being rodlike and terminally attached to the fibers. An explanation is, however, found in the fact, that all these chromosomes show a more or less clearly visible longitudinal split, which — as shown by the genesis of the chromosomes — is identical with the space between the two branches of a half ring.

In several cases this split extends all through the daughter chromosomes, so that they lose their V-shape, the two halves being quite separate from each other (chrom. 34, p. 63).

In the telophase the vacuoles are as a rule formed at the side of such a double chromosome or between two neighboring ones.

Later Cleavage Divisions.

(*a*) *Seven and One Half to Eleven Hours After Fertilization.*

In the prophase of the later cleavage divisions we miss the cross-shaped stage of the chromosomes. They appear within the nucleus as longitudinally split ribbons (earl. proph., p. 62); and at the time of attachment to the spindle fibers they are, without exception, V- or horseshoe-shaped, being attached by their middle point.

The appearance of the later stages is, up to about nine hours after fertilization, very much like that of the early cleavage, the chromosomes retaining their prophase shape, until the daughter chromosomes are separated.

After this time, however, the aspect of the metaphase is changed through a tendency in the two arms of the V-shaped chromosomes to approach (chrom. 12 (26), earl. anaph., p. 63).

(*b*) *Eleven to Fifteen and One Half Hours After Fertilization.*

The change just mentioned, proceeds rapidly, and fifteen and one half hours after fertilization the metaphase of the mitosis has an appearance very much like that of the second maturation division. The 28 chromosomes, having in the prophase passed through a V-shaped stage (chrom. 9, 16, later proph., p. 62), are in the metaphase forming as many terminally attached tetrads, standing stiffly out from the spindle (chrom. 12).

Most of these tetrads show a considerable thickening of their four elements at their inner end; and a comparison with later stages of the mitosis makes it probable, that this phenomenon is in some causal connection with a dislocation of the point of attachment to the fibers. (See below, p. 74.)

In accordance with the rodlike shape of the chromosomes no open rings were found in the early anaphase. The doubleness of the daughter chromosomes is, however, still often indicated by narrow openings between their two branches during the separation of the daughter chromosomes (chrom. 28, earl. anaph., p. 63).

In the later anaphase no longitudinal split was visible in the daughter chromosomes.

These are the main facts observed in the material of *Nereis* now in my hand. The series of stages is, however, not yet complete, and I therefore prefer to postpone a general discussion of the bearing of my results, until I have had an opportunity of examining the nature of the mitosis also at the end of the germ track, and of comparing the chromosomes of *Nereis* with those of some other types.

On this occasion I only wish to draw the conclusions reached through the examination of the maturation and cleavage division in *Nereis*, and also to point out some questions, the answer to which will be of importance for an understanding of the chromosome relations in this species.

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1. As already mentioned, the behavior of the chromosomes in all divisions in question is practically the same. Their point

of attachment, their plane of division, their typical changes of form during the separation of the daughter chromosomes and the longitudinal split of these chromosomes — these are characters common to all the divisions, taking place within fifteen hours after fertilization.

And yet, every division (or group of divisions) has its characteristic appearance, each representing one step in a series of transformations following the stage of the conjugation of the chromosomes.

The first maturation division is characterized by a great variability in the shape of the chromosomes and also in the time of the separation of the daughter chromosomes. No stage in this division can be considered as the metaphase of the whole mitotic figure, each chromosome passing through its characteristic stages independently of all the others, and without being placed in a typical equatorial plate. Characteristic also is a peculiarity in the (chemical or physical?) structure of the chromosomes, making them appear less stiff and consistent than in other divisions.[1] Thus the daughter chromosomes are by their separation very often sharply bent, their ends forming right angles with each other (chrom. 18–25, 1st mat. div., p. 63), and there is also a marked tendency towards a spherical shape of the free ends of the chromosomes.

Taking the first maturation division as a starting point, all the changes in the mitotic figures going on within 15–16 hours after fertilization may be looked upon as a gradual return from these irregularities to the normal mitosis.[2]

The chromosomes regain slowly their original more rigid structure; and their cross-shape during the separation of the daughter chromosomes becomes less conspicuous to the same degree as the rigidity increases.

Of great interest are also the gradual changes of the chromo-

[1] Meves (1897) and Kingsbury (1902) have drawn quite similar conclusions from the form of the daughter chromosomes in the first maturation mitosis of amphibians.

[2] The second maturation division does not form a good link in this series of transformations, its whole appearance being more like the later cleavage-divisions than the earlier ones. This is, however, a natural consequence of the lack of a resting stage before this division, the changes of the chromosomes within the nucleus being of importance for their appearance during the mitosis.

somes in the early prophase. The prophase of the first maturation mitosis is in most objects characterized by a more or less complete separation of the longitudinal halves of the chromosomes — and in *Nereis* this separation may take place along both longitudinal splits of the original tetrads. Also with regard to this character we find a gradual return to the general type. In the nuclei of the early blastomeres we still find a divergence of the four arms of the tetrad, although here without the variety in form characteristic of the first maturation division. Through an approach of each two arms of the prophase-crosses, the V-shaped chromosomes of the metaphase arise; and in later cleavage-divisions the V-shaped chromosomes of the early prophase are transformed into rod-like tetrads through a similar approach of their arms.

2. Another result of general interest, reached through a comparison of the maturation and cleavage divisions in *Nereis*, concerns the point of attachment of the spindle fibers to the daughter chromosomes.

Although it seems, that the first connection between chromosomes and fibers always takes place at homologous points of the chromosomes, there is a very strong evidence in favor of the assumption that this point is changed during the mitosis.[1]

In the cleavage divisions all the chromosomes are derived from the cross- or V-shaped chromosomes of the prophase, being attached at their middle point. If, therefore, the point of attachment is a fixed one, a subterminal attachment of the daughter chromosomes would seem absolutely excluded. According to the degree of opening of the longitudinal split the attachment of the daughter chromosomes might be called a median or a terminal one, but any intermediate attachment would seem impossible. And yet, we almost always find some of these chromosomes subterminally attached — not so often in the early cleavage as in the maturation and in the later cleavage divisions, where more than half of the daughter chromosomes are often found to be subterminally attached (see p. 63).

An indication of the way in which this dislocation of the fibers

[1] This probability was, from another point of view first suggested by Schreiner, 1906*b*, p. 433.

takes place is given in the later cleavage divisions. The terminal attachment of the tetrads in the metaphase is identical with the median one of the V-shaped chromosomes in the prophase. The tetrads are, however, as it were, pressed against the fibers of the spindle, their proximal ends being thickened or curved (p. 62). The point of attachment of the fibers is in this way changed from a terminal into a subterminal one, the thickened ends of the tetrads being found again as the short branch of the subterminally attached daughter chromosomes. In some cases it seems as if the point of attachment during the early anaphase is still further removed from the end of the chromosome — a dislocation probably due to the fact, that the median part of the daughter chromosomes makes less resistance against a separation than the ends. (See the triangular chrom.; sec. mat. div., p. 63).

3. What is the bearing of the chromosome-relations in *Nereis* with regard to the assumption of a universally existing reduction division?

In the papers of Schreiner (1904, 1906*a* and *b*), Grégoire (1905) and Montgomery (1905) the universality of a prereductional mode of maturation is based upon the similarity between the chromosomes of different species, and more especially on the general appearance of a "heterotypical" mitosis in the first maturation division.

Grégoire (1905) in his valuable review of the literature concerning the maturation divisions in plants and animals, tries to explain the phenomena in question as following his "hétérohoméotypique" scheme — the first maturation division being a heterotypical, the second a homeotypical one.

His provisional definition of these two modes of mitosis and his opinion with regard to their bearing is found in the following sentences (*loc. cit.*, p. 254).

"Pour la période qui nous occupe, la caractéristique de l'hétérotypie consiste dans la division longitudinale anaphasique; la caracteristique de l'homéotypie réside en ce que les chromosomes-filles de cette cinèse sont preparés dès la cinèse précédente par une division longitudinale."

". . . ce schéma, . . ., s'oppose directement au processus *postréductionnel*, mais il laisse ouverte la question du processus *prérèductionnel* et du processus *eumitotique*."

(P. 362): " Disons-le dès maintenant, c'est le *schéma préréductionnel* dont nous espérons démontrer la réalité."

A. and K. E. Schreiner who in their main results fully agree with Grégoire, characterize the heterotypical appearance of the first maturation division in *Tomopteris* as follows (1906*a*, p. 44): " Geht man . . . auf eine genauere Betrachtung des Verhaltens der Chromosomen in den Reifungsteilungen und auf einen Vergleich desselben mit dem Verhalten der Chromosomen in anderen Teilungen ein, so kann es ja keinem Beobachter entgehen, dass die I Reifungsteilung bei fast allen Objekten, wo es gelungen ist, die Struktur der Chromosomen zu analyzieren, einen gemeinsamen Typus zeigt, der sich von dem Typus aller anderen Teilungen in charakteristischer Weise unterscheidet; und zwar besteht der Unterschied darin, dass sich die Schwesterelemente der einzelnen Chromatinportionen bei dieser Teilung schon lange vor dem Eintreten der Mitose . . . in weiter Ausdehnung von einander trennen und während der ganzen Pro- und Metaphase eine viel gröszere Selbständigkeit zeigen, als in irgend einer anderen Teilung der Fall ist. Auch sind die Verbindungen zwischen den Schwesterelementen in dieser Teilung von ganz anderer Art als bei allen anderen Teilungen." . . . " Es scheint uns, dass das Auftreten dieser eigenthümlichen Bilder der Chromosomen während der ersten Teilung nach dem Eintreten der Zahlenreduktion nur dadurch befriedigend erklärt werden kann, dass hier Ganzchromosomen, die nie miteinander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

These considerations represent, according to the authors, the main reasons for their assumption of a prereductional maturation in *Tomopteris*, as it " allein aus der Betrachtung der verschiedenen Längsteilungen kaum möglich (ist) zu einer endgültigen Lösung dieser Frage zu gelangen."

Their assumption of a universality of this mode of maturation, they base upon the general appearance of the " *Tomopteris*-typus " also in other species. So much weight is laid upon this similarity, that the heterotypical character of a few chromosomes is considered a sufficient proof of a reductional nature of the mitosis. Thus in their description of the maturation divisions of *Myxine*, we find (1906*b*, p. 459):

"Nach der Einstellung in die Teilungsebene zeigen die Chromosomen in gewissen Fällen die für die I Reifungsmitose so charakteristischen, in die Äquatorialebene fallenden Verdikkungen und seitlichen Ausläufer, die sicher beweisen, dass es die Spalthälften der bivalenten Schlingen, die Konjuganten, sind, die hier getrennt werden."

The above cited sentences of Grégoire and Schreiner contain the view which forms the basis in their generalizations with regard to the reduction division.

Judging the chromosome relations in *Nereis* from the same point of view, we should find a reduction of the chromosomes taking place not only in each maturation division but also in each of a whole series of cleavage divisions. The early separation of the daughter chromosomes, the heterotypical shape of the chromosomes in metaphase, and their doubleness in the anaphase, are characters common to all these divisions.

This result is so clear and its consequences are so evident, that a further discussion upon this point would seem unnecessary. Before the question about the nature of the maturation divisions can be considered ripe for new generalizations, it will be necessary to widen the base of our investigations to a comparative study not only of the maturation process itself, but also of the changes of the chromosomes during the time following this period.

Though, however, the main base of the modern generalizations is removed through the knowledge of the chromosome relations in *Nereis*, it is not therefore excluded, that a reduction division may exist in this species as well as in others; and we now finally turn to the question:

4. How is the maturation process in *Nereis* to be understood?

In answering this question three different possibilities must be considered, all of which have already been applied for the maturation process in other species.

(*a*) One of the maturation divisions is a reductional one, separating chromosomes, which have conjugated at an earlier period.

(*b*) Both maturation divisions are to be considered as ordinary mitoses, the conjugating chromosomes having fused completely with each other (Boveri, 1890).

(*c*) The conjugating chromosomes do not separate again; but their fusion may proceed so slowly that the appearance of the following divisions is influenced by the doubleness of the chromosomes (Bonnevie, 1905, '06).

Of these three possibilities only the first one will be treated fully in this paper.

According to the concordant results of modern investigators the two longitudinally split halves of the chromosomes in the prophase of the first maturation mitosis (*a* — *a*, p. 62) are to be considered as two conjugating chromosomes united to form a bivalent one.[1]

If, therefore, these two halves were separated from each other in the first maturation division, then this division must with great probability be considered as a reductional one, and all the similar structures in the following divisions would have to be explained in some other way.

Such a conclusion might, in *Nereis*, as in so many other objects, easily be drawn, if the chromosomes of the early prophase are compared with those of the early anaphase; it would, indeed, seem very natural to consider the thickening in the equator of metaphase (or anaphase) chromosomes as identical with that connecting their two halves in the early prophase. But between these two stages there is another, the stage in which the chromosomes are first attached on the young spindle,[2] showing that the long axes of the chromosomes are placed at right angles to the axis of the spindle, that they are attached to the fibers at the connection point between their two halves and divided in a plane represented by their longitudinal split, and finally, that their position parallel to the spindle fibers is secondary — reached during the separation of their daughter chromosomes.

If, therefore, the assumption is correct, that each half of the chromosomes of the prophase represents one of the conjugating chromosomes, then the same must be true of the daughter chro-

[1] It makes here no difference, whether the conjugation of the chromosomes is considered as a parallel one or as having taken place "end to end"; in each case the connection between the two conjugates is supposed to be of the same kind at a stage directly preceding the maturation divisions.

[2] As will be shown in my final paper, there is no escape from this fact through the suggestion that I should have "confondu les stades" (Gregoire, 1904, p. 307).

mosomes; and *the first maturation division is certainly not a reduction division.*

The possibility, however, of the second maturation division of *Nereis* being a reductional mitosis, is not absolutely excluded, although the only reason for accepting this view must, as far as I can see, be sought in a preconceived assumption in favor of such a mode of maturation.

The chromosomes of the prophase show in the second maturation division, as in the first, and in the early cleavage divisions, two longitudinal splits, one of which is identical with the split of the daughter chromosomes of the first division, the other being a new formation generally not appearing till in the prophase of the second but sometimes indicated as early as in the anaphase of the first division. In most cases it seems impossible to decide with absolute certainty which of these splits represents the division plane of the chromosomes.

Supposed, however, that a separation of the conjugated chromosomes should take place in this division, then the thorough resemblance in the genesis and the whole appearance of the chromosomes during the maturation and cleavage divisions must be considered as a mere chance. The longitudinal split of the daughter chromosomes, would have a different meaning in each of the maturation divisions and in the cleavage; in the first maturation division it would mean the split between the conjugated chromosomes, in the second it must be explained in some other way — most likely, perhaps, as a precocious splitting for the next division; in the cleavage divisions, finally, the longitudinal split is certainly not to be seen in connection with the following division as it is shown to be identical with the space between the two branches of the V-shaped chromosomes of the prophase. In the same way all the other stages in the development of the chromosomes would, in spite of their detailed resemblance, have to be explained in different ways.

Considering, on the other hand, the fact that in this species, so favorable for an examination of the chromosomes, no evidence at all is found, which might establish a proof of the existence of a reduction division — it seems to me more natural to look upon the conformity in the behavior of the chromosomes as an expres-

sion also of a corresponding series of changes going on within them during each mitosis.

Whatever, therefore, the meaning may be of the different structures of the chromosomes, they ought, I think, to be looked upon from one and the same point of view in each of the maturation divisions as in the cleavage, and the results reached in any of these divisions may be used for an explanation of similar structures in the others.

According to this view, I consider the question of a reduction division in *Nereis* as lying outside of the actual discussion until a sufficient proof for its universality is established in other species.

The questions to be settled with regard to *Nereis* concern the two other possibilities, mentioned above — whether or not the conjugating chromosomes have fused completely before the maturation divisions.

As I said before, I have not yet the material for a definite answer of this question ; and it will be the aim of my following investigations to procure such material through a comparative study of the chromosomes of the germ track in *Nereis* and those of other species.

With our present knowledge of the chromosome-relations in *Nereis* the evidences in favor of each of these views seem to balance each other.

If we take our starting point in the prophase of the first maturation division, considering the two longitudinally split halves (*a* and *a*, pp. 62–63) of the chromosomes as the conjugates being terminally attached to the spindle, then it would seem natural to look upon the quite similarly shaped chromosomes of the following divisions from the same point of view. The gradual changes in the whole appearance of the mitosis within the first 15 to 16 hours after fertilization must then be considered as the expression of a slowly proceeding fusion of the conjugating chromosomes (*a* and *a*), their tendency of a divergence during the prophases gradually decreasing, and their fusion during the anaphases becoming always more complete.

If on the other hand, we begin with the early cleavage divisions, where all the chromosomes are V- or horseshoe-shaped and with a median attachment to the fibers — then it would seem

more natural to consider the longitudinal split of the anaphases merely as an unusually narrow space between the two arms of V-shaped daughter chromosomes, and the cross-shaped chromosomes of the prophases as arisen through a precocious separation of the daughter chromosomes, connected or crossing each other on their middle point. The same view must be held also with regard to the maturation and the later cleavage divisions, the tetrads, so often met with, being considered as merely morphological structures, arisen through an approach of the two arms of a V, that is, their two longitudinally split halves, *a* and *a* must be looked upon as forming one continuous ribbon, sharply bent on its middle point. The point of attachment of the chromosomes would, from this point of view, in all divisions be a median one, the many cases in which a terminal attachment seems to be shown, being explained as artefacts.

As will be seen from the above, each of these interpretations meets with difficulties, which at the present state of our knowledge interferes with the acceptation of any of them.

In order to solve these difficulties it will be of great importance to follow the changes in the mode of attachment of the chromosomes throughout the whole germ track — and especially to compare the chromosome-relations of species in which a median attachment of the chromosomes seems to be predominant (*Tomopteris*, *Salamandra*, etc.), with those of other species, in which the chromosomes are terminally attached to the fibers.

In my following papers I shall publish my first results of such a comparative study, and even if the solution of the problem of maturation is still far away, I hope to be able to throw some new light on the question.

COLUMBIA UNIVERSITY,
March, 1907.

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TO THE PLATES, PP. 62–63.

The chromosomes represented in these plates are selected from among a great number of camera drawings which will be published in a following paper.

They are all drawn to the same scale, enlargement about 3,500 : 1.

The chromosomes of the first maturation division are continuously numbered ; some of these numbers being applied also to chromosomes of the later divisions, showing essentially the same structure as those of the first.

The letters *a* and *a* are applied to the morphologically distinguishable halves of the chromosomes, demonstrating their reappearance in each group of divisions, but without any interpretation concerning the meaning of these structures.

The relatively larger size of the chromosomes of the later cleavage divisions (7 to 15 h. aft. fertil.) may be due to their fixation in Flemming's fluid, while those of the maturation and the early cleavage are fixed in picro-acetic acid.